k-ecoli457: A genome-scale *Escherichia coli* kinetic metabolic model satisfying flux data for multiple mutant strains

Ali Khodayari* (auk241@psu.edu) and **Costas D. Maranas**

Department of Chemical Engineering, Pennsylvania State University, University Park, Pennsylvania, USA.

http://www.maranasgroup.com

Project Goals: The goal of this effort is to construct a genome-scale kinetic model of *Escherichia coli* metabolism by making use of Ensemble Modeling (EM) concepts. Model parameterization is carried out using multiple flux datasets for different substrates and growth (aerobic vs. anaerobic) conditions.

Kinetic models of metabolism at a genome-scale that faithfully recapitulate the effect of multiple genetic interventions would be transformative in our ability to reliably design novel overproducing microbial strains. Here, we introduce k-ecoli457, a genome-scale kinetic model of *Escherichia coli* metabolism that satisfies fluxomic data for wild-type and 25 mutant strains under different substrates and growth conditions. Model k-ecoli457 contains 457 reactions, 337 metabolites and 295 substrate-level regulatory interactions. Parameterization is carried out using a genetic algorithm by simultaneously imposing all available fluxomic data (about 30 measured fluxes per mutant). Model predictions were tested against multiple experimentally measured datasets that were not used during model parameterization. These included (i) 898 steady-state metabolite concentrations for twenty of the mutant strains [1-4], (ii) 234 Michaelis-Menten constants (185 K_m and 49 k_{cat} values) from BRENDA and EcoCyc, and (iii) 320 literature reported product yields for designed strains covering 24 different bioproducts. Comparisons revealed that 66% of the predicted metabolite concentrations as well as 51% and 63% of the estimated K_m and k_{cat} values, respectively, are within the experimentally reported ranges. Notably, the average relative error of k-ecoli457 predictions for the product yield in 129 out of 320 designed strains is within 20% of the measured values. Stoichiometric model based techniques such as FBA, minimization of metabolic adjustment (MOMA) or maximization of product yield were within 20% of the experimentally reported yield for only 16, 18 and 65 of the designed strains, respectively. Overall, the predicted product yields by k-ecoli457 achieve significantly higher value of correlation with experimental data (i.e., Pearson's correlation coefficient of 0.84) than FBA, MOMA or maximization of product yield (i.e., 0.18, 0.37 and 0.11, respectively). These

results quantitatively demonstrate that k-ecoli457 can reliably be used to predict genetically perturbed *E. coli* phenotypes under different growth conditions with a substantially higher accuracy than any other earlier modeling effort (k-ecoli457 is available for download at http://www.maranasgroup.com).

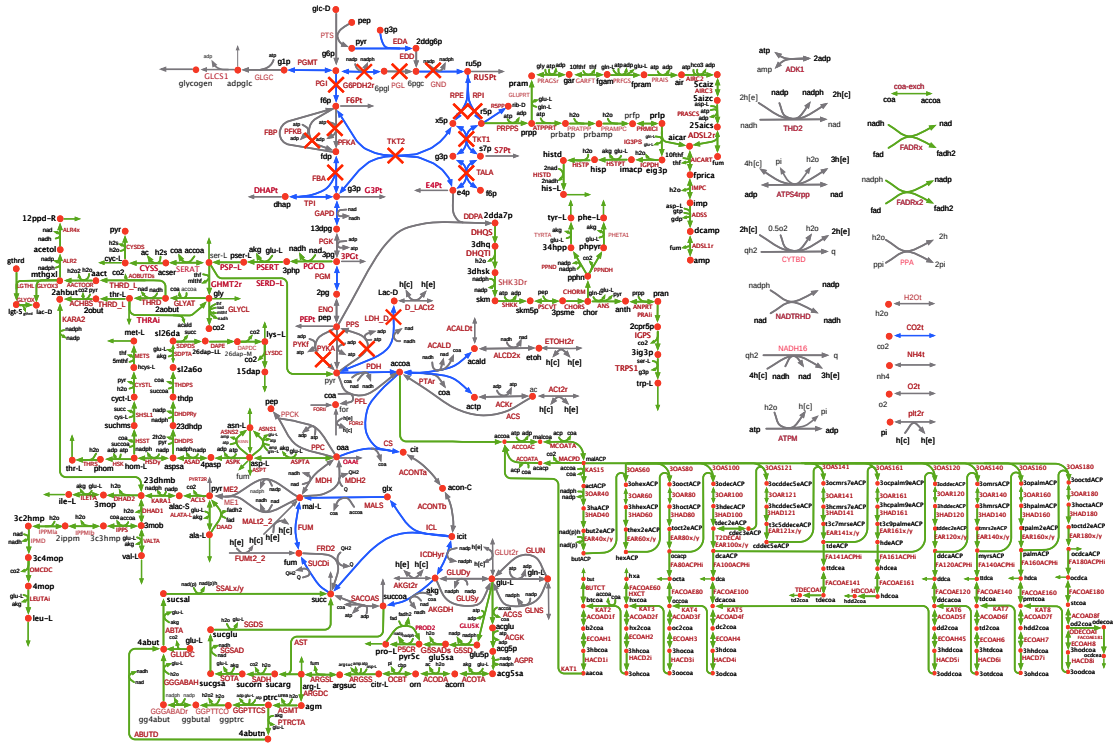

Figure A pictorial representation of the k-ecoli457 model of *E. coli* metabolism. Red X's denote the location of reaction deletions in the mutant datasets. Reactions in the previously developed core model [5] are shown in grey (no flux data) and blue (with flux data) while the additional reactions in k-ecoli457 are shown in green.

The work was supported by the genomic science grant from Department of Energy, USA (grant# DE-SC0012377).

References

1. Ishii N, Nakahigashi K, Baba T, Robert M, Soga T, Kanai A, Hirasawa T, Naba M, Hirai K, Hoque A *et al*: **Multiple high-throughput analyses monitor the response of E. coli to perturbations**. *Science* 2007, **316**(5824):593-597.
2. Kabir MM, Ho PY, Shimizu K: **Effect of ldhA gene deletion on the metabolism of Escherichia coli based on gene expression, enzyme activities, intracellular metabolite concentrations, and metabolic flux distribution**. *Biochem Eng J* 2005, **26**(1):1-11.
3. Zhao J, Baba T, Mori H, Shimizu K: **Global metabolic response of Escherichia coli to gnd or zwf gene-knockout, based on 13C-labeling experiments and the measurement of enzyme activities**. *Applied microbiology and biotechnology* 2004, **64**(1):91-98.
4. Zhao J, Shimizu K: **Metabolic flux analysis of Escherichia coli K12 grown on 13C-labeled acetate and glucose using GC-MS and powerful flux calculation method**. *Journal of biotechnology* 2003, **101**(2):101-117.
5. Khodayari A, Zomorrodi AR, Liao JC, Maranas CD: **A kinetic model of Escherichia coli core metabolism satisfying multiple sets of mutant flux data**. *Metabolic engineering* 2014, **25**:50-62.